



Growth of Desulfovibrio vulgaris Hildenborough Wild Type, Fur, PerR, and Zur Mutants, and Its Lysogenic Phages

Huei-Che Bill Yen, Kelly Bender, and Judy D. Wall



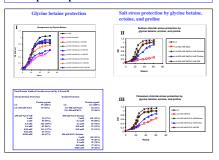




Introduction:

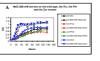
- 1. Studies of the basic growth physiology and morphology of D. vulgaris under normal and stress conditions.
- 2. Investigation of the endogenous phage of D. vulgaris.

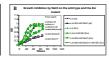
1. Salt stress and glycine betaine, ectoine, and proline protection



2. Salt Stress of the wild type, Fur, Per, and Zur mutant

A. The Fur and Per mutants are more sensitive to salt stress B. The Zur mutant is more resistant to salt stress.





Salt stress Conclusions

- 1. Glycine betaine, ectoine, and proline protect against NaCl and KCl str sorbital stress.
- ate protects salt stress slightly.
- 3. The following chemicals do not protect against salt stress: Choline, Glycine, S (Methyl glycine), Dimethylglycine, Glycerol, Aspartate, and Trytophan.

 4. The salt stress and glycine betaine protection are no different under iron limi

- The lactate consumption rate of the wild type was 0.58 mM lactate/ug protein.
 The consumption rates for NaCl and KCl stressed cultures were 0.77 and 0.71 mM
- lactate/ µg protein, respectively.

 6. The Fur and PerR mutant are more sensitive to salt stress, but the Zur mutant is mo

7. EM Pictures



LS (60 mM Lac and 30 mM SO.=)

















Formate/Sulfate (120 mM/40 mM)









250 mM NaCl





























8. D. vulgaris Phages





us phage released from D vulgaris post

200-400 PFU/ml filtrate

stationary phase cultures

tant (Lactate/sulfate=60/30 mM) over 10⁴ PFU/ml

9. Lysogenic strains of DP4

Plaque assay of the lysogenic strains of DP4 (PFU/ml)***							
	L88-33	L88-66	L88-17	DP4	Dv		
0 hr*	(50)		0	0	0		
7 hr	0	6,400	10,000	0	1,000		
18 hr	0	16,000	28,000	0	(100)		
31 hr	0	35,000	28,000	0	(8,000)		
42 hr	(300)	26,000	51,000	0	(40)		
70 hr**		0		0	0		
Hours after adding Mitomycin C at 28 hours. Controls, no Mitomycin C added. Images were estimated by counting 1/8 of a niate. A total of 2 or 3 obstess were counted.							

3. Growth inhibition by metals and others

A. Metal concentration needed to completely inhibit the growth of D. vulgaris for at least 150 hours in Yen45 medium (60 mM lactate and 30 mM sulfate) at 37°C:

1.	K,CrO ₄ :	0.7 mM	2. Na,SeO ₄ :	3 mM	3. Na,SeO ₄ : 0.3 mM
4.	Na ₂ MoO ₄ :	0.2 mM	5. K ₂ TeO ₃ :	0.1 mM	6. CuCl ₂ : 0.8 mM
7.	CoCl ₂ :	15 mM	8. ZnCl ₂ :	4 mM	 MnCl₂: 40 mM

B. Inhibitory concentration (slow down the growth rate, delay the growth for at least 60 hours, or inhibit growth for at least 100 hours):

ı.	5-Fluorouracil:	0.2 µg/ml	2. L-Cysteine:	12 mM
3.	5-Fluoroorotate:	200 μg/ml	4. L-Cystine:	6 mM
5.	8-Aza-2,6-Diaminopurine:	40 μg/ml	Sodium sulfide:	50 mM
١.	8-Aza-guanine:	7 µg/ml	8. Uranyl nitrate:	4 mM
).	Sodium nitrate:	100 mM	10. G418:	200 μg/ml
	Sodium nitrite:	2 mM	12. Kanamycin:	700 μg/ml
13.	Carbenicillin:	400 μg/ml	14. Tetrcycline:	30 μg/ml
15.	Ampicillin:	400 µg/ml	16. D-Cycloserine:	6 mM

4. Sensitivity to oxidizing agents of the wild type, Fur, PerR and Zur mutants

	Wild type (Kelly) MIC (mM)	Fur mutant (JWK707) Sensitivity***	PerR mutant (JWK708) Sensitivity	Zur mutant (JWK709) Sensitivity
1. H ₂ O ₂	$3 \mathrm{mM}$	very sensitive	sensitive	
2. Butyl hydroperoxide	3 mM	very sensitive	very sensitive	
3. Menadione*	7-10 mM	more sensitive	sensitive	
4. Methyl viologen*	0.2 mM	very sensitive	sensitive	
5. Benzyl viologen®	0.06 mM	more sensitive	sensitive	sensitive
6. N-ethylmaleimide*	1.5 mM	more sensitive	sensitive	
7. Diamide**	>40 mM	>40 mM	>40 mM	

- Redox Cycling Agents
- Thio Oxidizing Agent Sensitivity; Sensitivity to the oxidizing agent comparing to the wild type. NG; No growth for 150 hours or longer

5. Growth inhibition of the Fur mutant by metals

- 1. The Fur mutant is more sensitive than the wild type to the following metals: ZnCl2, TeO3, SeO4 (PerR is more sensitive to Zn and TeO3)
- 2. The Fur mutant is more resistant than the wild type to the following metals: MnCl₂, CoCl₂, SeO₃ (Zur is more resistant to MnCl₂)
- 3. No different between the wild type and the Fur mutant to the following metals:











4. D. vulgaris is more resistant to TeO3 and SeO4 in the medium containing 0.3 mM Cysteine.

Examples:







Growth response of the wild type, Fur, PerR, and Zur mutants to medium pH

There is no growth difference at pH 5.5.

A. There is no growth difference at pH 5.5.

B. The Fur. PerR and Zur mutants are more resistant to pH 10.







Conclusions:

- 1. Glycine betaine, ectoine, and proline protect both NaCl and KCl stresses.
- The Fur and the PerR mutants are more sensitive to salt stress than the wild type, but the Zur mutant is more resistant.
- 3. The Fur and PerR mutant are more sensitive to many oxidizing agents and metals than the wild type. 4. Wild-type cells are elongated under certain nutrient unbalance, salt stress, and
- 5. D. vulgaris releases phages spontaneously from post stationary culture and
- lysogenic strains of DP4 can be isolated at a rate of about 10%.